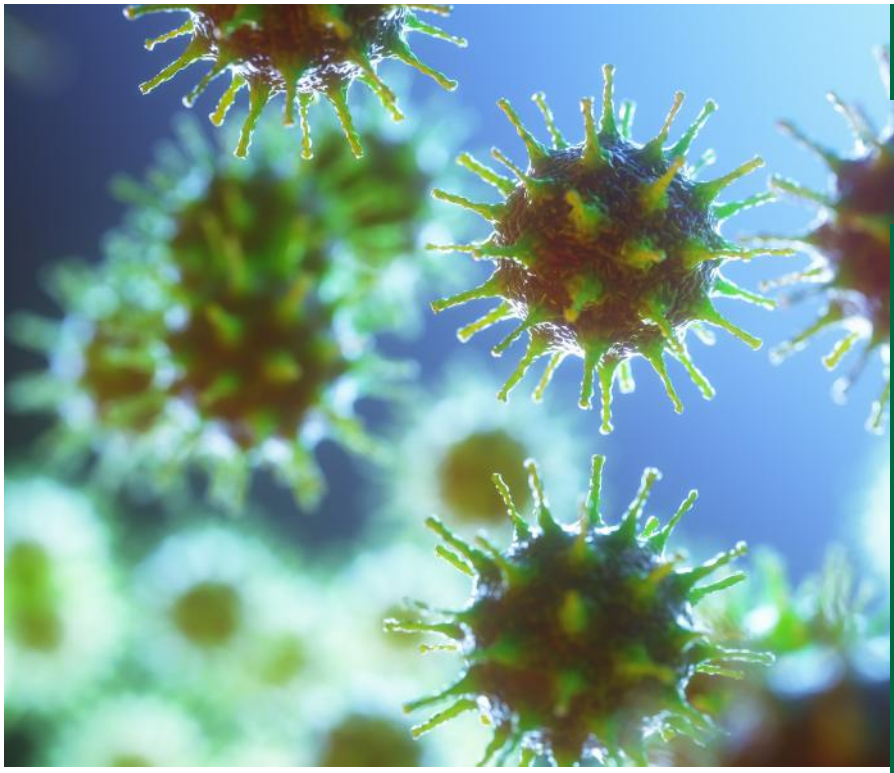

APPLICATION BRIEF

Liquid Chromatography

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HPLC Analysis of Lopinavir and Ritonavir Using a Quasar C8 Column

Lopinavir and ritonavir are commonly prescribed as a fixed-dose combination drug therapy for the treatment of Human Immunodeficiency Viruses (HIV) and belong to a class of antiviral drugs known as protease inhibitors. Lopinavir by itself has insufficient bioavailability to be an effective therapy. However, ritonavir inhibits cytochrome P450 enzymes in the liver known to break down lopinavir, increasing its concentration in the blood to its desired therapeutic window. [1] Lopinavir and ritonavir treatment as a single medication is listed by the World Health Organization's (WHO) list of essential medicines, which serves as a model of the safest and most effective medicines required by a health system.[2] Early studies have shown promising results as a treatment against SARS-CoV-2 and further research is currently being conducted into its efficacy.[3]

This application brief describes the use of a Quasar C8 column for the analysis of lopinavir and ritonavir (Figure 1).

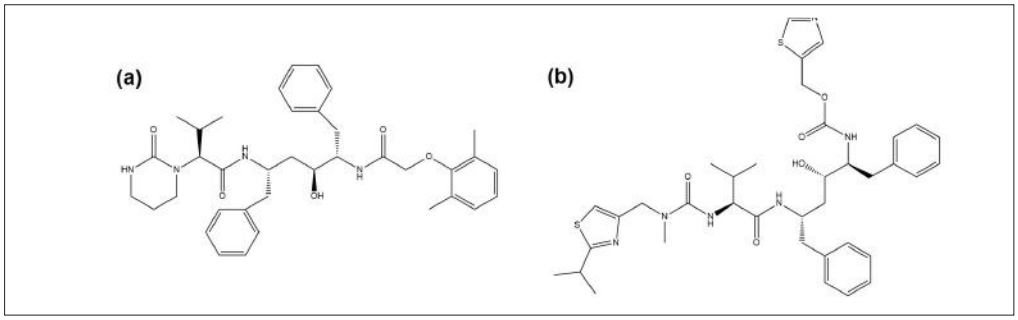

Figure 1. Chemical structures of lopinavir (a), and ritonavir (b).

Experimental Conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters.

Instrument	PerkinElmer Flexar™ with PDA Plus™ Detector			
Quasar C8	150 mm	4.6 mm	5 µm	N9308880
Mobile Phase	**A:** 4.1 g/L monobasic potassium phosphate in water **B:** Acetonitrile **45% A, 55% B**			
Flow Rate	1.5 mL/min			
Temp	40°C			
Wavelength	215 nm			
Injection Volume	50 µL			
Analytes	Lopinavir and Ritonavir			

Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 0.22 µm nylon filter, P/N 02542881.

A 4.1 g/L monobasic potassium phosphate buffer was prepared in de-ionized (DI) water. In addition, a 0.1 mg/mL stock standard solution each of lopinavir and ritonavir was prepared in 50/50 acetonitrile/buffer. From this standard, a working standard solution (0.025 mg/mL each of lopinavir and ritonavir) was prepared in 50/50 acetonitrile/buffer.

Results and Discussion

Ritonavir and lopinavir have been successfully separated in just under six minutes using a Quasar C8 (150 x 4.6 mm, 5 µm) column (P/N: N9308880), as demonstrated in Figure 2. The Quasar C8 column is ideally suited to the analysis of small molecules, such as ritonavir and lopinavir, whilst providing excellent efficiency (calculated using the tangential method) for and peak shape. This is due to Quasar's optimized ligand bonding technology and ultra-high purity silica base, which minimizes unwanted silanol interactions. Table 2 provides a summary of the peak parameters obtained for the two compounds. Asymmetry was calculated at 10 % peak height and provided values of 1.13 and 1.12 for ritonavir and lopinavir, respectively. Relative standard deviations (RSD %) of the peak areas for each compound were calculated using data from five replicates. The results showed excellent levels of precision, with RSDs ≤ 0.2 % (Table 2). Additionally, the two compounds showed good resolution, achieving a value of 2.68. Resolution of 1.5 or greater (baseline resolved) is desired as it ensures that the compounds are well separated and allows accurate determination of peak area and/or peak height.

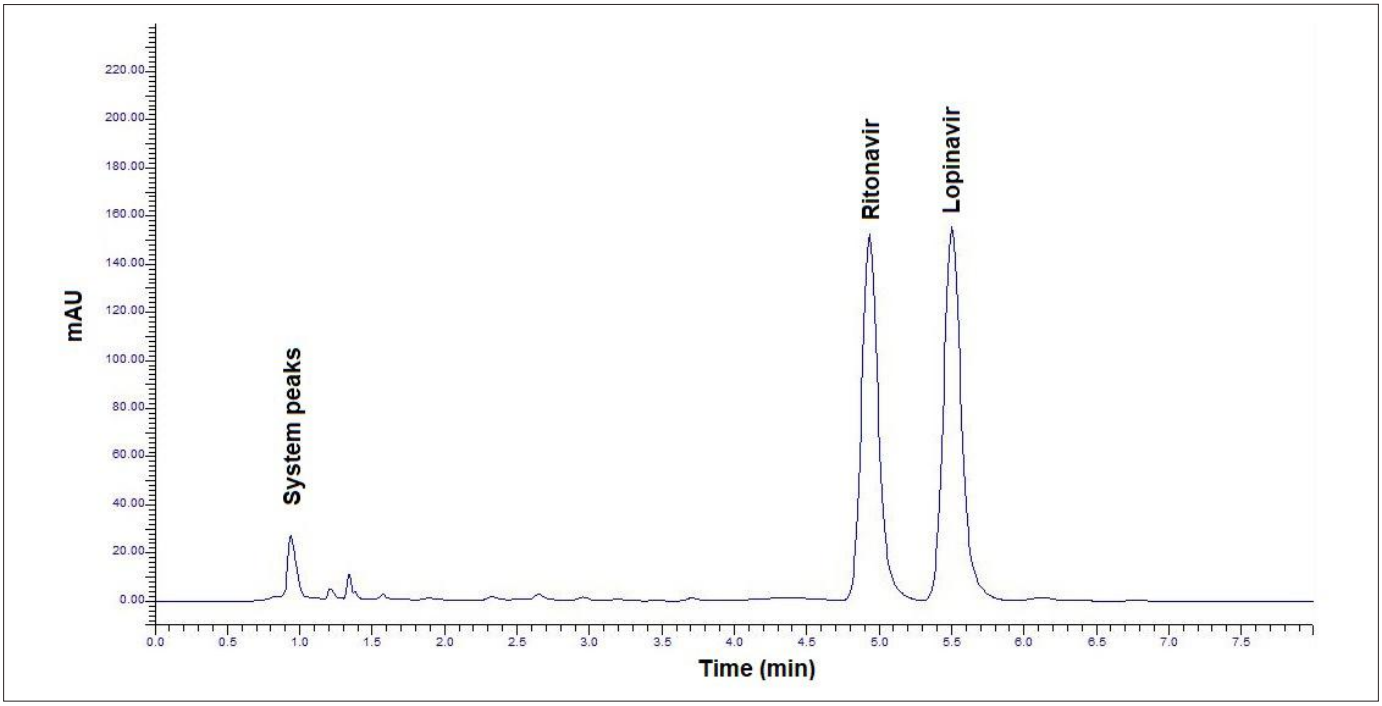

Figure 2. Analysis of ritonavir and lopinavir using a Quasar C8 column (150 x 4.6 mm, 5 µm).

Table 2. Results summary.

Parameter	Compound	
	Ritonavir	Lopinavir
Retention Time (min)	4.93	5.50
Tailing Factor	1.13	1.12
Relative Standard Deviation of Peak Area (%)	0.15	0.20
Efficiency (N)	8761	9600
Resolution	2.68	

Conclusion

- The Quasar C8 HPLC phase offers a repeatable and efficient separation of ritonavir and lopinavir.
- The ultra-high purity silica base and low residual silanol activity yields excellent peak shape.
- Analysis is rapid, with both compounds eluting in under six minutes. Run time could be further reduced by using a shorter Quasar C8 column, with a smaller particle size.

Consumables

Component	Description	Part Number
HPLC Column	Quasar C8 (150 x 4.6 mm, 5 µm)	N9308880
HPLC Vials	2 mL amber 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (polypropylene) Cap with PTFE/ Silicone pre-slit Septa (100/pack)	N9306203
Syringes	Syringe 1 mL BD Luer-Lok Disposable, Pack of 100	02542890
Syringe Filters	0.22 µm nylon filter	02542881
PEEK Fittings	Finger-tight for 1/16" OD PEEK tubing	09920513

References

1. D. J. Kempf, *Comprehensive Medicinal Chemistry II,* Elsevier, Amsterdam, 2007.
2. WHO Model List of Essential Medicines, https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?ua=1, (accessed 27/03/2020).
3. Fang Liu, et. Al, *International Journal of Infectious Diseases,* March 2020, pre-proof, https://www.sciencedirect.com/science/article/pii/S1201971220301326, (Accessed 27/03/2020).